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Ultrastructure and Dynamics of Selective Mitochondrial Injury in Carcinoma Cells After Doxycycline Photosensitization In Vitro

CHRISTOPHER R. SHEA, MD,*
DIANA WHITAKER, MS,†
GEORGE F. MURPHY, MD,† and
TAYYABA HASAN, PhD*

From Wellman Laboratories of Photomedicine, Massachusetts General Hospital, Department of Dermatology, Harvard Medical School, Boston, Massachusetts and the Department of Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania†*

Mechanisms and intracellular sites of photosensitized damage were investigated in cultured MGH-U1 cells treated with doxycycline (DOTC). Cells were examined by phase-contrast, fluorescence, and electron microscopy at various times (15 minutes to 24 hours) after ultraviolet irradiation (320–400 nm). DOTC localized selectively within the mitochondria, as shown by colocalized fluorescence with rhodamine 123 (R123). Photosensitization at 1 J/sq cm caused striking swelling of the mitochondrial matrix and disruption of the cristae, accompanied by loss of the ability of mitochondria to concentrate R123. These changes progressed during the first hour after irradiation, and then were followed by partial recovery of mitochondrial ultrastructure and function. At no time were other organelles seen to be affected. It appears that this selective, photosensitized alteration was a consequence of localized and partially reversible damage to the mitochondrial inner membrane. In contrast, exposure to 2–6 J/sq cm caused irreversible injury and necrosis. (Am J Pathol 1988, 133:381–388)

TETRACYCLINES (TCs) are among the most commonly prescribed medications. An important side effect of TC therapy is photosensitivity,[1] but the subcellular lesions and morphologic changes associated with this response are not well characterized. In eukaryotic cells TCs generally localize selectively within mitochondria[2] and in the absence of light can specifically inhibit mitochondrial protein synthesis;[3] mitochondria might, therefore, be a logical target for TC-sensitized photochemical reactions as well. In previous studies[4] of cultured human bladder carcinoma cells treated with the potent photosensitizer, doxycycline (DOTC), the authors demonstrated that an early consequence of exposure to long-wave ultraviolet radiation (UVA, 320–400 nm) was loss of the ability of mitochondria to concentrate the cationic, fluorescent probe rhodamine 123 (R123). This functional defect was accompanied by the formation of cytoplasmic vacuoles, which might represent hydropic mitochondria. Enhanced permeability of the plasma membrane to ethidium bromide, as detected by fluorescence microscopy, occurred only several hours after mitochondrial injury was evident and required higher radiant exposures (≥2 J/sq cm). The photosensitized damage therefore appeared to be at least partially selective for mitochondria.

The possibility of damage to other structures, however, could not be excluded by studies on the light-microscopic level. Indeed, other investigators have shown direct damage to the plasma membrane of erythrocytes after TC photosensitization at high radiant exposures,[5,6] and the reported association of TC with ribosomes[7,8] and nuclei[9] raises the possibility of multiple sites of action of TC-sensitized photochemis-

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Address reprint requests to Tayyaba Hasan, PhD, Wellman Laboratories of Photomedicine, Massachusetts General Hospital, Department of Dermatology, Harvard Medical School, Boston, MA 02114.

try in cells. In this report, electron microscopy has been used to study the sequence of ultrastructural changes in DOTC-photosensitized cells. In addition, the patterns of localization of R123 in these cells as a function of radiant exposure and time following irradiation have been studied. The goals of these experiments were: 1) to define the ultrastructural targets of DOTC phototoxicity; 2) to study the dynamics of vacuolation, to address the possibility of recovery from photosensitized damage; and 3) to correlate ultrastructural changes with mitochondrial function, as assessed by the pattern of R123 fluorescence in photosensitized cells.

Materials and Methods

Cells

MGH-U1 cells,[10] an adherent line derived from a human transitional-cell carcinoma of the bladder, were maintained as exponentially-growing stock cultures at 37 C in a humidified, 95% air: 5% CO_2 atmosphere, using McCoy's 5A medium with HEPES buffer (Gibco Laboratories, Life Technologies, Inc., Grand Island, NY), supplemented with 5% fetal bovine serum (Gibco). For experiments, cells were harvested by treatment for 5 minutes with 1% trypsin-EDTA (Gibco) and inoculated in complete medium into plastic Petri dishes at a density of 6×10^4 cells/sq cm. Experiments were performed 24 hours after plating, when cells had formed a subconfluent monolayer.

Irradiation Procedure

Cells were incubated at 37 C for 30 minutes in 20.7 μM (10 μg/ml) DOTC (Sigma Chemical Co., St. Louis, MO) in Dulbecco's phosphate buffered saline (DPBS) (Gibco) containing 0.9 mM $CaCl_2$ and 0.49 mM $MgCl \cdot H_2O$ at pH 7.2. They were then immediately irradiated at room temperature (24 C) with broad-spectrum UVA from a filtered xenon-arc lamp as described previously.[4] Irradiance was between 7 and 19 mW/sq cm, as measured by a filtered, cosine-corrected photodiode radiometer (International Light, Inc., Newburyport, MA); there was no significant heating at the irradiances used. Radiant exposures used were 1, 2, and 6 J/sq cm; control cultures received either UVA alone, DOTC alone, or neither. After experimental treatment, cells were washed twice in DPBS and replaced into complete medium for incubation until observation or fixation.

Electron Microscopy

Cells were processed for electron microscopy immediately after irradiation and at 10, 30, 60, 90, 120, and 210 minutes, and 24 hours. Cells were treated with 0.05% trypsin and 0.53 mM EDTA and centrifuged for 10 minutes at 1500*g*; the resulting pellets were washed by centrifugation with DPBS and then fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4, containing 5 mM $CaCl_2$ for 45 minutes at room temperature. The cells were then washed three times by centrifugation with cacodylate buffer, post-fixed for 1 hour with 1% OsO_4 in cacodylate buffer, washed again twice, pelleted into a 2% aqueous solution of agar, dehydrated in graded (up to 70%) ethanols, and stained *en bloc* with 2% uranyl acetate in 70% ethanol for 45 minutes. After sequential dehydration in absolute ethanol and in propylene oxide, the cell pellets were embedded in an epon-araldite mixture. Thin sections were cut on a Porter Blum MT2B ultramicrotome, stained with uranyl acetate and lead citrate, and viewed with a JEOL JEM 100CX II electron microscope.

Fluorescence Microscopy

At various times after irradiation, as given below, cultures were incubated for 15 minutes at 37 C in 10 μM R123 (Eastman Kodak, Rochester, NY) in DPBS, washed twice in DPBS, replaced into complete medium, and viewed with a ×40 water-immersion objective on a Zeiss phase-contrast and epifluorescence microscope. For viewing DOTC fluorescence, excitation with 320–380 nm and emission greater than 420 nm were used; for viewing R123 fluorescence, excitation with 450–490 nm and emission of 520–560 nm were used. There was no cross-excitation of the two fluorescent probes with either filter combination. Cells were observed at 15, 30, 45, 60, 75, 90, and 105 minutes, and 2, 3, and 4 hours after irradiation. At each time, the presence or absence of vacuolation was determined by phase-contrast viewing, and the pattern of fluorescence of DOTC and R123 (*ie,* cytoplasmic *vs.* mitochondrial localization) was assessed.

Results

Electron Microscopy

Control cells (Figure 1A, B) were characterized by rounded contours with occasional membrane ruffles and microvillous processes. The nuclei were peripherally displaced, frequently exhibited indented contours, and showed peripheral aggregation of heterochromatin. Numerous organelles were present throughout the cytoplasm. Mitochondria were characterized by smooth, ovoid contours, regularly-spaced cristae traversing the organelles perpendicular to their

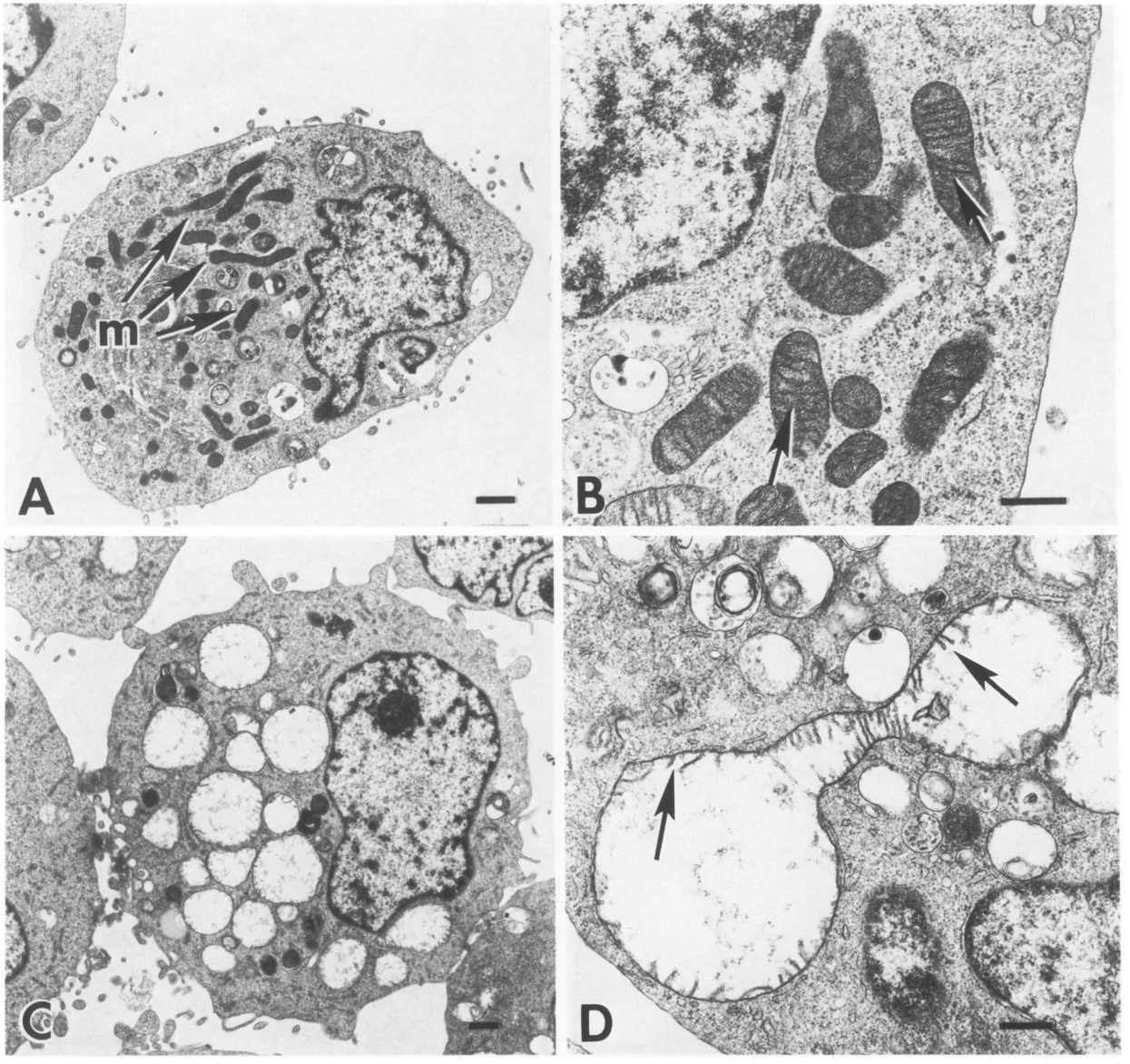

Figure 1A—Control cells exhibit infolded, peripherally-displaced nuclei and numerous ovoid mitochondria (m) throughout the cytoplasm. **B**—At higher magnification, the mitochondria exhibit intact cristae that periodically traverse these organelles perpendicular to their long axes (arrows). A moderately electron-dense matrix is uniformly distributed between the cristae. **C**—Cells harvested 30 minutes after treatment with DOTC (20.7 μM × 30 minutes) and UVA (1 J/sq cm) show striking enlargement of most mitochondria. Note that structures adjacent to the swollen mitochondria, including nucleus, cytoplasmic contents, and round, electron-dense lysosomes are not affected by this treatment. **D**—Higher magnification of an affected mitochondrion shows fragmentation of cristae (arrows) and aggregation of amorphous matrix material centrally. Bar represents 1 μ in panels A and C, and 0.5 μ in panels B and D.

long axes, and a moderately electron-dense, amorphous matrix between cristae. Other organelles included lysosomes, Golgi apparatus, rough endoplasmic reticulum, numerous ribosomes, and rare lipid droplets. Cells treated with UVA alone or DOTC alone were indistinguishable from control cells.

Treatment of cells with DOTC and UVA (1 J/sq cm) resulted in time-dependent alterations in the mitochondria of the majority of cells. These changes consisted of striking mitochondrial enlargement characterized by disruption of cristae, apparent partial dissolution and aggregation of residual electron-dense mitochondrial matrix, and marked distortion of the mitochondrial contours. These changes were not apparent in cells fixed immediately after irradiation but became progressively pronounced in samples fixed 10, 30, and 60 minutes later (Figure 1C, D). Remarkably, organelles and cytoplasmic matrix immediately adjacent to altered mitochondria were completely unaffected; at no time was there generalized swelling of the DOTC-photosensitized cells at this radiant exposure. By 1.5 hours after irradiation, affected mitochondria were generally smaller than at 10–60 minutes after irradiation, although some remained sig-

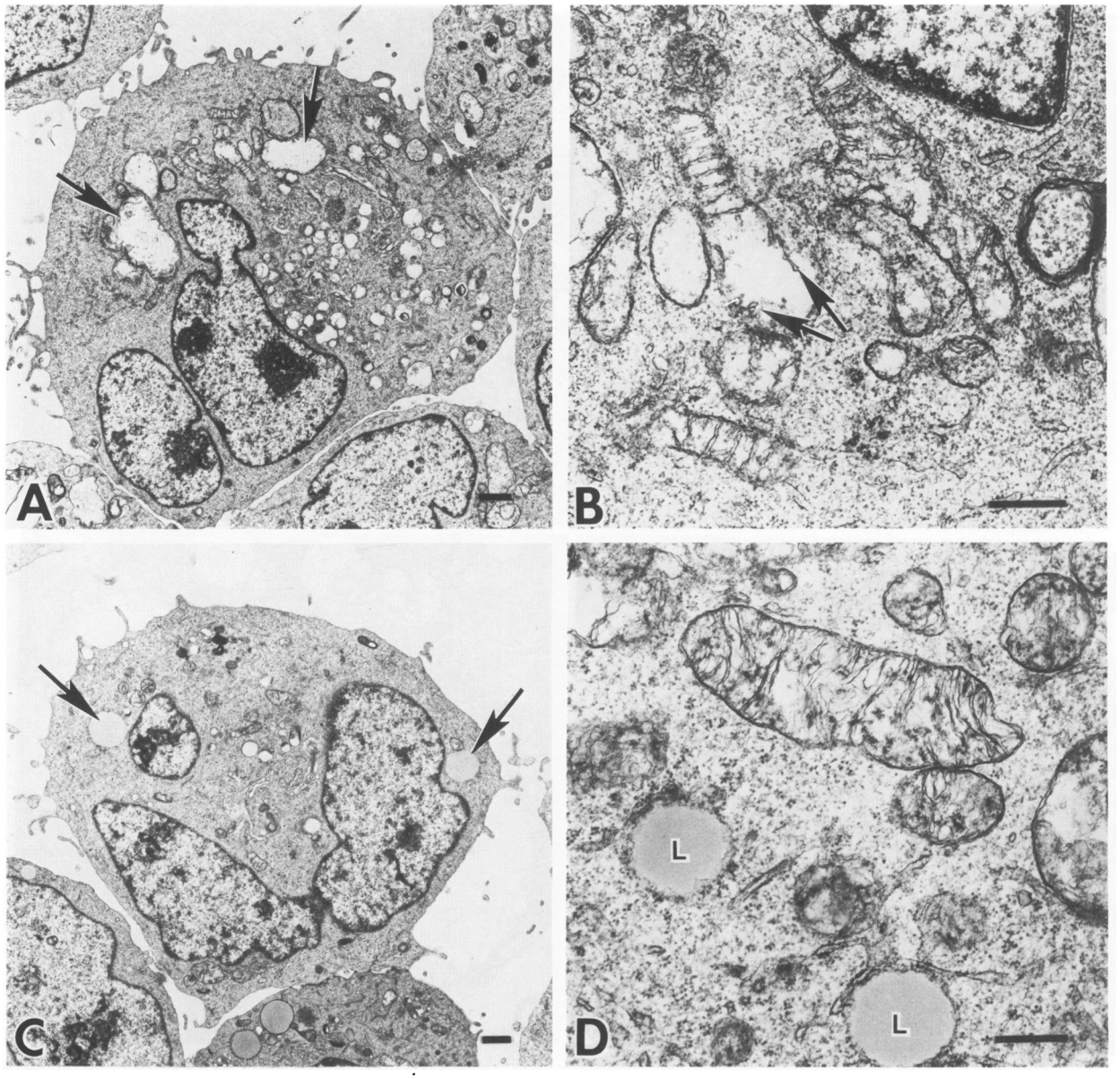

Figure 2A—Two hours after treatment with DOTC (20.7 μM × 30 minutes) and UVA (1 J/sq cm), variable mitochondrial enlargement is detected (arrows). **B**—At higher magnification some cristae have apparently reconstituted, while others remain disrupted (arrows). **C**—Twenty-four hours after treatment mitchondrial enlargement is not apparent, although the absolute number of mitochondria appears diminished. **D**—By this juncture, lipid bodies have begun to form within the cytoplasm (arrows) and are characterized by zones of relatively electron-lucent amorphous material not bound by enclosing membranes (L). Note that, although mitochondria have reassumed normal size, their shape remains slightly irregular, cristae are incompletely reformed, and electron-dense, amorphous matrix material remains unevenly distributed. Bar represents 1 μ in panels A and C, and 0.5 μ in panels B and D.

nificantly enlarged and internally disrupted (Figure 2A). The apparent reconstitution of many mitochondria was accompanied by foci where cristae had apparently reformed, juxtaposed with zones of persistent disruption (Figure 2B). By this juncture, the Golgi apparatus was prominent, numerous lysosomes were observed, and membrane debris and myelin figures were observed in many cells. Those mitochondria not exhibiting reconstitution were generally swollen and exhibited nearly complete disruption of internal structure. Mitochondrial reconstitution continued progressively, so that at 3.5 hours after treatment only rare cells contained swollen and disrupted mitochondria characteristically seen at 30 and 60 minutes. By 24 hours after treatment, the majority of cells contained mitochondria of normal size (Figure 2C). In most cells, however, the absolute number of mitochondria appeared decreased compared with control cells. At high magnification, reconstituted mitochondria exhibited persistent abnormalities consisting of focal irregularities of contour and uneven aggregation of electron-dense matrix material (Figure 2D).

Although the majority of cristae had reformed, the mitochondrial architecture was irregular and disorderly when compared with that of control cells. Lipid bodies were prominent within most cells at this time (Figure 2D), and were characterized by relatively electron-lucent, amorphous material not enclosed by limiting membranes. At no point were transitional structures showing features of both mitochondria and lipid bodies observed.

For determination of the effect of varying the radiant exposure on the ultrastructure of DOTC-treated cells, cultures were irradiated with 6 J/sq cm and sequentially studied. By 30 minutes after treatment, only occasional mitochondria were swollen; the majority were of normal size and shape, but were pale and diffusely less electron-dense than mitochondria of control cells. By 3.5 hours, the majority of cells exhibited marked degenerative alterations and focal cytolysis. By 24 hours, widespread necrosis characterized the cells. Thus, specific injury and apparent reconstitution of mitochondria at an ultrastructural level were not so apparent in cells treated with higher doses of UVA.

Fluorescence and Phase-Contrast Microscopy

Incubation of cells with DOTC and R123 in the absence of UVA irradiation caused no morphologic alterations detectable by phase-contrast microscopy (Figure 3A); the cells were adherent, had few vacuoles, and had abundant mitochondria, seen as particulate and tubular structures that were typically most numerous adjacent to one side of the nucleus. DOTC and R123 cofluoresced selectively within the mitochondria (Figure 3B, C); this colocalization was seen whether the cells were incubated with the two fluorophores simultaneously, or sequentially in either order. Cells irradiated with UVA in the absence of DOTC showed no alterations at any time.

Irradiation of DOTC-treated cells with 1 J/sq cm led to the appearance of numerous cytoplasmic vacuoles, correlating with the swollen mitochondria seen by electron microscopy, by 15–30 minutes after irradiation (Figure 4A); at these times uptake of R123 into mitochondria was impaired, and there was instead a diffuse, cytoplasmic localization of R123 fluorescence, tending to spare the vacuoles (Figure 4B). DOTC fluorescence was almost completely bleached by the irradiation, and its pattern of localization could not be clearly discerned. At progressively longer times after irradiation with 1 J/sq cm, phase-contrast microscopy showed fewer, but generally larger, vacuoles. By 2 hours after irradiation, vacuoles were rare and mitochondria could again be recognized by phase-contrast microscopy in many cells (Figure 4C); at this time, R123 uptake reverted to a predominantly mitochondrial pattern, although there was nonselective, cytoplasmic R123 fluorescence in some cells (mixed pattern, Figure 4D). Thereafter, the morphology and R123-localizing-pattern of the DOTC-treated cells irradiated with 1 J/sq cm did not change significantly at later times.

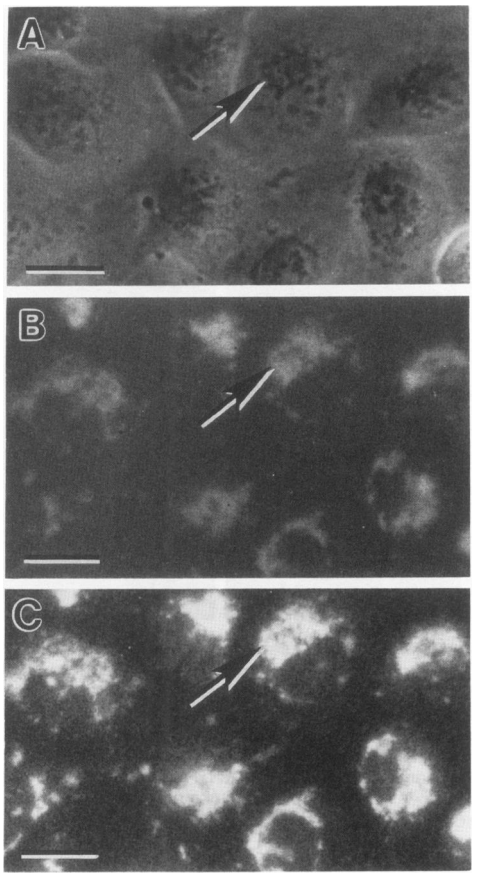

Figure 3A—Phase-contrast microscopy of unirradiated cells shows them to be adherent and free of large vacuoles. Mitochondria (arrow) are discerned as collections of particulate bodies disposed adjacent to nuclei. **B**—By fluorescence microscopy (excitation 320–380 nm, emission >420 nm) DOTC is shown to be localized selectively within mitochondria of these same cells. **C**—R123 colocalizes with DOTC, as shown by fluorescence microscopy of the same field (excitation 450–490 nm, emission 520–560 nm). Bar represents 10 μ.

As in the ultrastructural study, these responses of DOTC-treated cells varied as a function of radiant exposure. R123 assumed a cytoplasmic localization immediately after exposure of cells to 2 J/sq cm and 6 J/sq cm, but there was no reversion at later times to a mitochondrial or mixed pattern of R123 fluorescence at these higher radiant exposures; R123 fluorescence was persistently cytoplasmic from 15 minutes to 4 hours after irradiation (Figure 5). Cells irradiated with these higher radiant exposures showed a lesser degree of discrete vacuolation than at 1 J/sq cm, tending in-

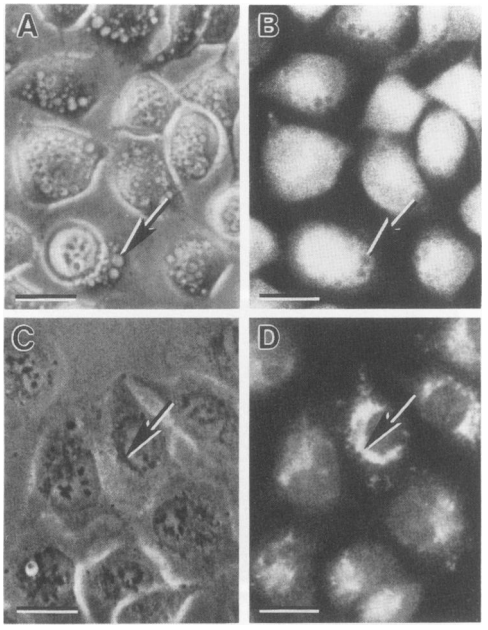

Figure 4—Time-dependent effects of DOTC (20.7 μM × 30 minutes) and UVA (1 J/sq cm). **A**—Thirty minutes after irradiation, multiple cytoplasmic vacuoles (arrow) representing hydropic mitochondria are present. **B**—In the same field R123 fluorescence is localized diffusely in the cells' cytoplasm and conspicuously spares the vacuoles. **C**—By 2 hours after irradiation, vacuoles have largely resolved, fluorescence microscopy of the same field shows R123 to be now localized mainly in the mitochondria (arrow). **D**—there is a background of cytoplasmic localization in some cells. Bar represents 10 μ.

stead to undergo progressive, generalized swelling, with rounding and retraction at 2 J/sq cm and ballooning at 6 J/sq cm.

Discussion

The present study demonstrates that DOTC is taken up selectively into the mitochondria of MGH-U1 cells, as assessed by colocalized fluorescence of DOTC with the specific mitochondrial probe R123. This finding is in accordance with many studies showing mitochondrial localization of TCs in kidney cells,[2] liver cells,[2,11] and lymphocytes;[12] one study reported a predominantly nuclear localization of TC but employed a high and probably toxic concentration.[9] The mechanisms of localization of TCs within mitochondria may be complex, possibly involving chelation of intramitochondrial calcium,[13] electrostatic attraction,[14] or affinity for the mitochondrial ribosomes.[15]

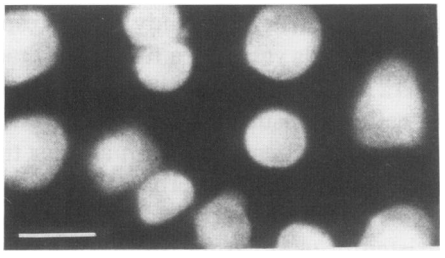

Figure 5—DOTC-treated (20.7 μM × 30 minutes) cells 4 hours after irradiation with 6 J/sq cm UVA. Vacuoles are absent; R123 fluorescence is cytoplasmic, with no tendency for mitochondrial localization. Most cells have undergone generalized swelling and are rounded. Bar represents 10 μ.

The highly organelle-selective distribution of DOTC in MGH-U1 cells forms the basis for mitochondrion-specific phototoxicity after UVA irradiation. Excited-state singlet oxygen ($^1O_2$) is an important mediator of TC-sensitized photochemical damage.[4,16] Based on the values in aqueous solution for the lifetime ($\sim 5 \times 10^{-6}$ seconds)[17] and diffusion constant (2×10^{-5} sq cm second^{-1})[18] of $^1O_2$, its maximal diffusion distance in water is approximately 0.2 μ; the lifetime in a nonpolar environment is significantly longer, but in a biologic milieu containing numerous possible substrates for oxidation reactions the diffusion of $^1O_2$ is still likely to be extremely limited. Selective localization of a $^1O_2$-producing chromophore thus can sensitize organelle-specific photochemical damage at a resolution comparable with that achieved with lasers by photothermal mechanisms using time-resolved[19] or microbeam[20] techniques. Not all $^1O_2$-mediated photosensitizers can cause such highly selective injury, however. For instance, ultrastructural studies of phototoxicity sensitized by hematoporphyrin and hematoporphyrin derivative (HPD) have shown lesions in a variety of subcellular structures, including mitochondria, endoplasmic reticulum, and plasma membrane.[21-24] This less selective pattern of injury may reflect the tendency of HPD to be partitioned nonselectively into membranes, the fact that it comprises a complex mixture of chemical species with different affinities for different cellular compartments, or the use of radiant exposures well above the threshold for damage, leading to a poorly-confined pathophysiologic cascade within porphyrin-photosensitized cells.

Mitochondrial swelling can occur as an artifact of fixation and other technical manipulations.[25] In the present study this is clearly not the case, because vacuolation occurs and resolves in an identical manner in living, adherent cells, and because mitochondria in glutaraldehyde-fixed control cells show no tendency to undergo swelling. The selectivity of mitochondrial alterations after DOTC photosensitization at 1 J/sq cm argues against their being a generalized, nonspecific response of membrane-bound organelles to cell injury (the cloudy swelling of classical pathology).[26] Mitochondrial swelling typically results from an influx of solutes and, by osmosis, of water into the matrix space secondary to increased permeability of the mitochondrial inner membrane. DOTC photosensitization may cause mitochondria to swell by a direct

photochemical reaction with inner-membrane constituents, or by indirectly causing changes in bioenergetics; these two mechanisms are in fact probably closely interconnected. TCs are relatively inefficient at sensitizing photohemolysis,[6] but this may reflect the lack of intimate association of TCs with the erythrocyte membrane[27] rather than an intrinsically poor ability to photosensitize membrane damage in general.

Preliminary work using intact, DOTC-treated MGH-U1 cells indicates that enzymes of the inner mitochondrial membrane are more susceptible to photoinhibition than are those located in the cytosol, mitochondrial matrix, or outer membrane;[28] specifically, the F_0F_1 ATPase and cytochrome C oxidase activities appear to be highly sensitive to DOTC-mediated photochemistry. Such enzyme inhibitions would act synergistically to deplete ATP, and thereby to diminish the activity of energy-dependent transport systems of the inner mitochondrial membrane, with consequent derangements of salt and water balance within the matrix. These effects may result from direct photosensitization by DOTC or from the toxicity of a photoproduct. One of the TC photoproducts characterized is a quinone;[29] quinones are known to interfere with proton transport and oxidative phosphorylation.[30] Alternatively, the mechanism of DOTC-photosensitized mitochondrial swelling may involve, in part, photochemically-induced changes in the ability of DOTC to chelate intramitochondrial calcium; resultant changes in Ca^{++}-dependent transport systems[31] could mediate osmotically significant shifts in salt concentrations across the inner membrane. Studies of swelling of isolated mitochondria after DOTC photosensitization under defined energetic and ionic conditions are planned to help clarify the physiologic basis of this response.

After exposure to 1 J/sq cm, there is progressive recovery of the swollen mitochondria over several hours; the reversal of swelling may involve activity of the Na^+/K^+ antiporter or of the anionic channel of the inner mitochondrial membrane.[32,33] This morphologic reconstitution is accompanied by a partial return of the mitochondria's ability to concentrate R123, implying resumption of bioenergetic functions and restoration of the transmembrane proton gradient.[14] This recovery is incomplete, however, for these cells persistently show an abnormal, mixed pattern of R123 localization and a perturbed mitochondrial ultrastructure. Cells at this state are viable but their proliferative and biosynthetic activities are diminished.[34] In contrast, DOTC-treated cells irradiated with higher radiant exposures (2–6 J/sq cm) undergo progressive and irreversible degeneration eventuating in death. These observations are consistent with the work of Takagi et al[35] in which generalized DMCT-photosensitized damage to L929 cells was reported at high radiant exposures (50 J/sq cm) and high drug concentrations (400 μM). It is notable that although these suprathreshold radiant exposures do cause mitochondrial injury as reflected in the cytoplasmic localization of R123, they induce less prominent mitochondrial swelling. Radiant exposures of 2–6 J/sq cm may damage the mitochondrial inner membrane so severely that it becomes highly permeable to flux of solutes in both directions, and therefore cannot generate sufficient osmotic forces for net water flux inward.

The swelling of mitochondria after DOTC photosensitization is similar to that occurring *in vivo* following a reversible ischemic insult. Mitochondrial swelling due to ischemia can increase following reperfusion,[36] a finding consistent with the postulated role of oxidative damage (eg, via superoxide) in the pathogenesis of postischemic injury.[37] In this regard it is noteworthy that TC phototoxicity is oxygen-dependent,[38] that TCs can photochemically generate $^1O_2$,[16] and that quenchers and enhancers of excited oxygen species can modify TC-sensitized phototoxicity.[4,38] Thus, DOTC-photosensitized and postischemic injury to mitochondria may follow a common pathophysiologic pathway. Mitochondrial injury induced *in vitro* by DOTC photosensitization is highly selective, reproducible, and dose-dependent; our experimental system may therefore provide an excellent model for the study of oxygen-dependent mitochondrial injury and recovery.

References

1. Blank H, Cullen SI, Catalano PM: Photosensitivity studies with demethylchlortetracycline and doxycycline. Arch Dermatol 1968, 97:1–2
2. DuBuy HG, Showacre JL: Selective localization of tetracycline in mitochondria of living cells. Science 1961, 133:196–197
3. Kroon AM, Dontje BHJ, Holtrop M, Van den Bogert C: The mitochondrial genetic system as a target for chemotherapy: tetracyclines as cytostatics. Cancer Lett 1984, 25:33–40
4. Shea CR, Wimberly J, Hasan T: Mitochondrial phototoxicity sensitized by doxycycline in cultured human carcinoma cells. J Invest Dermatol 1986, 87:338–342
5. Nilsson R, Swanbeck G, Wennersten G: Primary mechanisms of erythrocyte photolysis induced by biological sensitizers and phototoxic drugs. Photochem Photobiol 1975, 22:183–186
6. Bjellerup M, Ljung B: Photohemolytic potency of tetracyclines. J Invest Dermatol 1985, 84:262–264
7. Reboud AM, Dubost D, Reboud JP: Photoincorporation of tetracycline into rat-liver ribosomes and subunits. Eur J Biochem 1982, 124:389–396

8. Goldman RA, Hasan T, Hall CC, Strycharz WA, Cooperman BS: Photoincorporation of tetracycline into *Escherichia coli* ribosomes: Identification of the major proteins photolabeled by native tetracycline and tetracycline photoproducts and implications for the inhibitory action of tetracycline on protein synthesis. Biochem 1983, 22:359–368
9. Park JK, Dow RC: The uptake and localization of tetracycline in human blood cells. Br J Exp Pathol 1970, 51: 179–182
10. Lin C-W, Lin JC, Prout GR: Establishment and characterization of four human bladder tumor cell lines and sublines with different degrees of malignancy. Cancer Res 1985, 45:5070–5079
11. Zuckerman AJ, Baker SF, Dunkley LJ: The effect of tetracycline on human liver cells in culture. Br J Exp Pathol 1968, 49:20–23
12. Holland P, Sleamaker K: A cytologic evaluation of intracellular tetracycline fluorescence in normal and neoplastic lymphocytes. Acta Cytolog 1969, 13:246–249
13. Chandler DE, Williams JA: Intracellular divalent cation release in pancreatic acinar cells during stimulus-secretion coupling. II. Subcellular localization of the fluorescent probe chlorotetracycline. J Cell Biol 1978, 76:386–399
14. Johnson LV, Walsh ML, Bockus BJ, Chen LB: Monitoring of relative mitochondrial membrane potential in living cells by fluorescence microscopy. J Cell Biol 1981, 88:526–535
15. Kroon AM, Van den Bogert C: Antibacterial drugs and their interference with the biogenesis of mitochondria in animal and human cells. Pharmaceut Weekblad Sci Ed 1983, 5:81–87
16. Hasan T, Khan AU: Phototoxicity of the tetracyclines: photosensitized emission of singlet delta dioxygen. Proc Natl Acad Sci USA 1986, 83:4604–4606
17. Rodgers MAJ: Solvent-induced deactivation of singlet oxygen: Additivity relationships in nonaromatic solvents. J Amer Chem Soc 1983, 105:6201–6205
18. Tannock IF: Oxygen diffusion and the distribution of cellular radiosensitivity in tumors. Br J Radiol 1972, 45:515–524
19. Murphy GF, Shepard RS, Paul BS, Menkes A, Anderson RR, Parrish JA: Organelle-specific injury to melanin-containing cells in human skin by pulsed laser irradiation. Lab Invest 1983, 49:680–685
20. Berns MW, Rattner JB, Meredith S, Witter M: Current laser microirradiation studies. Ann NY Acad Sci 1976, 267:160–175
21. Moan J, Johannessen JV, Christensen T, Espevik T, McGhie JB: Porphyrin-sensitized photoinactivation of human cells in vitro. Am J Pathol 1982, 109:184–192
22. Coppola A, Viggiani E, Salzarulo L, Rasile G: Ultrastructural changes in lymphoma cells treated with hematoporphyrin and light. Am J Pathol 1980, 99:175–181
23. Kato H, Aizawa K, Shinohara H, Konaka C, Nishimiya K, Kawate N, Yoneyama K, Takahashi H, Saito M, Shyan HM, Tomono T, Kinoshita K, Yoshihama I, Hayata Y: Cytomorphological changes caused by hematoporphyrin derivative and photodynamic therapy. Lasers Life Sci 1986, 1:13–27
24. Klaunig JE, Selman SH, Shulok JR, Schafer PJ, Britten SL, Goldblatt PJ: Morphologic studies of bladder tumors treated with hematoporphyrin derivative photochemotherapy. Am J Pathol 1985, 119:236–243
25. Romert P, Matthiesen ME: Swelling of mitochondria in immersion-fixed liver tissue. Acta Anatom 1981, 109: 332–338
26. Ghadially FN: Ultrastructural pathology of the cell and matrix. 2nd ed. London, Butterworth's, 1982, 196–203
27. Van den Bogert C, Dontje BHJ, Kroon AM: The antitumor effect of doxycycline on a T-cell leukemia in the rat. Leukemia Res 1983, 9:617–623
28. Thomas AC: Deoxytetracycline-photosensitized inactivation of mitochondrial enzymes in human bladder carcinoma cells. Honors Thesis, Department of Biochemistry, Harvard University, 1986
29. Davies AK, McKellar JF, Phillips GO, Reid AG: Photochemical oxidation of tetracycline in aqueous solution. J Chem Soc Perkin Trans 1979, 2:369–375
30. Rossi L, Moore GA, Orrenius S, O'Brien PJ: Quinone toxicity in hepatocytes without oxidative stress. Arch Biochem Biophys 1986, 251:25–35
31. Matlib MA: Action of bepridil, a new calcium channel blocker on oxidative phosphorylation, oligomycin-sensitive adenosine triphosphatase activity, swelling, Ca^{++} uptake and Na^{+}-induced Ca^{++} release processes of rabbit heart mitochondria *in vitro*. J Pharmacol Exp Ther 1985, 233:376–381
32. Garlid KD, Bearis AD: Evidence for the existence of an inner membrane anion channel in mitochondria. Biochem Biophys Acta 1986, 853:187–204
33. Sorgato MC, Keller BU, Stuhmer W: Patch-clamping of the inner mitochondrial membrane reveals a voltage-dependent ion channel. Nature 1987, 330:498–500
34. Hasan T, Wimberly J, Shea CR: Phototoxicity of deoxytetracycline and amiodarone to human lymphocytes and the T-cell line HPB-ALL (abstr). Photochem Photobiol 1985, 41:59S
35. Takagi S, Michiko Y, Numata T, Mizuno N: Ultrastructural changes in cultured cells induced by demethylchlorotetracycline (DMCT) phototoxic reaction with emphasis on initial changes. Photomed Photobiol 1985, 7:27–28
36. Jennings RB, Schaper J, Hill ML, Steenbergen C, Reimer KA: Effect of reperfusion late in the phase of reversible ischemic injury: Changes in cell volume, electrolytes, metabolites, and ultrastructure. Circ Res 1985, 56:262–278
37. Jolly SR, Kane WJ, Bailie MB, Abrams GD, Lucchesi BR: Canine myocardial reperfusion injury: Its reduction by the combined administration of superoxide dismutase and catalase. Circ Res 1984, 54:277–285
38. Hasan T, Kochevar IE, McAuliffe DJ, Cooperman BS, Abdulah D: Mechanism of tetracycline phototoxicity. J Invest Dermatol 1984, 83:179–183

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